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Research Article

Antinociceptive and Anti-depressant like Activities of Methanolic Flower Extract of *Nymphaea nouchali*

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Abstract: The aim of this study was to evaluate the antinociceptive and neuropharmacological activities of methanol extract of *Nymphaea nouchali* (Nymphaeaceae) flower (MENN).The antinociceptive activity of MENN was evaluated by heat induced (tail immersion test) and chemical induced pain models (acetic acid-induced writhing). The effect of MENN on central nervous system (CNS) was studied using hole cross test, open field test. MENN showed strong, significant and dose-dependent antinociceptive activity in both acetic acid-induced writhing and tail immersion test a tall experimental doses (200mg/kg and 400mg/kg). Acetic acid induced writhing test revealed that the extract at the lower dose inhibited59.97% and at the higher dose produced a maximum of 64.75% inhibition of writhing that is comparable to the reference drug Diclofenac Sodium. MENN also showed reduced locomotor activity in both hole cross and both open field tests. So, it is evident that MENN possesse strong antinociceptive activity as well as CNS depressant activity. The results justify the ethnomedicinal use of *N. nouchali* flower in different painful conditions and CNS disorders.
Keywords: *Nymphaea nouchali*, antinociceptive, neuropharmacological, CNS depressant activity

INTRODUCTION

From ancient times, plant parts have been used successfully to cure and prevent diseases throughout history [1]. According to World Health Organization (WHO) 80% of the earth's inhabitants depend upon traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components [2]. Most of the phytomedicines refer to the use of any plant's roots, leaves, bark ,fruits, seeds, berries, or flowers for medicinal purposes by greater percentage of people [3]. *Nymphaea nouchali* (synonym: *Nymphaea stellate*), is a perennial aquatic herb belonging to the family Nymphaeaceae. It is commonly known as the white water lily. It is an important and well-known medicinal plant, widely used in Ayurveda and Siddha system of medicines for the treatment of diabetes, liver disorders, inflammation and urinary disorders and as a bitter tonic. The fruit of this plant is globose containing round, flask shaped seeds. The seeds are used as stomachic and restorative. The seeds are also prescribed as diet for diabetes in the ayurvedic system of medicine [4]. Previous studies have revealed that seeds possess significant antioxidant [5], antidiabetic [6] and antibacterial and antifungal [7], antihepatotoxic activity [8]. Phenols, tannins, glycosides, flavonoids, saponins and alkaloids are also reported in the seeds [5]. Lately, a new steroid, nymphasterol, has been isolated and identified from the seeds [9].

N.nouchali is the national flower of Bangladesh and commonly known as "Shapla" in Bengali. *N. Nouchali* is a large perennial aquatic herb with short round rhizomes[7, 10]. *N. Stellata* is commonly known as Indian blue water lily / Indian water lily in English and has different vernacular names in India. Sometimes this water lily is often referred as 'blue lotus of India which is known as *N. nouchali*. The Indian system of medicines, particularly Ayurveda and Siddha, uses *N. stellata* as a single drug or in combination with other drugs [10]. From the survey of Ethnobotanical survey of the Tripura tribe of Bangladesh revealed that root cluster of *N. nouchali* has been used for Urinary problem, burning sensations in urinary tract, Burm var. leucorrhea in women [11]. Beside this previous study showed the extract of *N. nouchali* have significant and moderate membrane stabilizing activity based on different extraction. Also chloroform and aqueous soluble fractions of methanol extract revealed significant antibacterial and antifungal activities [7]. Different solvent extracts of the entire plant have shown the presence of saponins, tannins,

sterols, alkaloids, and flavonoids. Strong Antidiabetic Activity has been reported by *N. stellata.* No tumor Inhibition, significant reduction of antihepatotoxicity and moderate Cholinergic Activity has been observed by *N. stellata*, [10, 12, 13]. The extract also has been reports as strong analgesic activity [14].

METHODS AND MATERIALS

Plant material and extraction

The flowers of *N. nouchali* were collected from Siddeswari, Dhaka in July 2009. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no. 34370) for future reference. The flowers were extracted with 80% of methanol in a soxhlet apparatus at an elevated temperature. The extract was concentrated by evaporation under reduced pressure at 40^0C using Buchi rotary evaporator to have gummy concentrate of greenish color extract.

Chemicals and drugs

The following drugs and chemicals were used in the current study: Diclofenac sodium, Morphine and Diazepam (Square Pharmaceuticals Ltd., Bangladesh), acetic acid (Merck, Germany), methanol (Merck, Germany), Tween 80 (India).

Animals

For the experiment Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 g, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). Animals were maintained under standard environmental conditions (temperature: 24.0±1.0°C, relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

Phytochemical screening

The methanol extract of *N. nouchali* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Dragendorffs reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce suds. Gum was tested using Molish reagents and concentrated sulphuric acid. These were identified by characteristic color changes using standard procedures [15].

Acute Toxicity Test

The mice were divided into control and three test groups each containing five animals. MENN was administered to the animals orally at doses of 1000, 2000, and3000mg/kg. The mice were given access to food and water *adlibitum* and all animals were observed for allergic symptoms and mortality for the next 72 h [16].

Antinociceptive Test

Acetic Acid-Induced Writhing Test

Antinociceptive response of the extract of *N. nouchali* (200 and 400 mg/kg) was assessed by counting number of writhes (constriction of abdomen, turning of trunk and extension of hind legs) induced by 0.7% acetic acid solution in mice [17]. Number of writhes per animal was counted during 15 min test period, beginning 3 min after the injection of acetic acid. Diclofenac sodium 50 mg/kg b. wt was used as a reference drug.

Tail immersion test

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice [18]. The animals were treated as discussed above. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 , 90 and120 min after the administration of drugs. Morphine 25 mg/kg was used as a reference drug.

Neuropharmacological Test

Hole cross test

The method was adopted as described by Takagi *et al.* [19]. A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drugs.

Open field test

This experiment was carried out as described by [20]. The animals were divided into control and test groups containing five mice each. The test group received *N. nouchali* extract at the doses of 200 and 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water).The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30,60, 90, and 120min after oral administration of the test drugs.

STATISTICAL ANALYSIS

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group; $p < 0.05$, 0.001 were considered to be statistically significant.

RESULT

Phytochemical screening

Phytochemical screening of the crude extract of *N. nouchali* showed the presence of alkaloids, flavonoids, glycosides, carbohydrates, saponins, sreroids and tannins (Table-1).

Table 1: Preliminary Phytochemical Investigation of MENN

Phytochemical Test	Name of the tests	Result
Test for Carbohydrate	Molisch's test	Present
Test for Reducing Sugar	Benedict's test	Absent
	Fehling Test	Present
Test for Glycosides	General test	Present
Test for Alkaloids	General Laboratory test- Mayer's test Hager's test Wagner's test Dragendorff's Test	 Absent Present Present Present
Test for Flavonoids	General Test	Present
Test for Tannins	Ferric chloride test	Present
Test for resins	General test	Absent
Test for saponins	Frothing test	Present
Test for proteins	Millon's test	Absent
Test for steroids	General Test	Present

MENN=Methanol extract *N. nouchali* flower.

Acute Toxicity Test

It was also found that acute oral administration of MENN at the doses of 1000, 2000 and 3000mg/kg did not produce any allergic manifestations or mortality during the observation period of 72 h after administration. Therefore, it is conceivable that MENN may not be toxic at our experimental doses up to 3000mg/kg.

Acetic Acid-Induced Writhing Test

Methanolic extracts of *N. nouchali* were found to exhibit good analgesic activity at 400mg/Kg dose level. The methanolic extract produced 59.97% and 64.75% writhing inhibition at oral doses of 200 mg/kg and 400mg/kg body weight of mice whereas the standard Diclofenac produced 78.09 % writhing inhibition in mice (Table-2).

Table 2: Effect of MENN on Acetic acid induced writhing in mice

Treatment	Dose (mg/kg)	Mean ± SEM	% of Inhibition
Vehicle	-	50.2 ± 1.53	0.00
Diclofenac sodium	10mg/kg	11 ± 1.87**	78.09
MENN	200mg/kg	20.1 ± 3.82*	59.97
MENN	400mg/kg	17.7 ± 4.46 **	64.75

Each value is presented as mean ± SEM (n = 5), MENN=Methanol extract *N. nouchali* flower. **p <0.001, *p <0.05 compared with the control group (Dunnett's Test).

Tail immersion test

The tail withdrawal reflex time following administration of the extract of *N. nouchali* was found to increase with increasing dose of the sample. The result was statistically significant ($p<0.05$- 0.001) and was comparable to the reference drug Morphine (Table-3).

Table 3: Analgesic effect of MENN in Tail Immersion Test

Treatment	Dose (mg/kg)	Observation				
		Pre-treatment	30 min	60 min	90 min	120 min
Vehicle	-	2.2 ±0.375	3.20 ±0.580	2.6 ± 0.250	3.400 ±0.510	3.2 ±0.510
Morphine	25 mg/kg	3.2 ±0.375**	8.06 ±0.270**	9.12 ± 0.225**	8.54 ±0.410**	6.14±0.410**
MENN	200 mg/kg	3.482±0.33	3.55±0.615	2.5± 0.235*	2.6± 0.20	2.1± 0.20*
MENN	400 mg/kg	3.22±0.66	3.62±0.545	3.96±0.36*	3.6± 0.56	3.22± 0.56

Each value is presented as mean ± SEM (n = 5), MENN=Methanol extract *N. nouchali* flower. **p <0.001, *p <0.05 compared with the control group (Dunnett's Test).

Hole cross test

The number of hole crossed from one chamber to another by mice of the control group is increased from 30 minutes to 90 minutes (table-4). Hole cross test of *N. nouchali* 200mg/kg & 400mg/kg dose showed significant decrease of movement from its initial value at 0 to 120 minutes and they fell asleep.

Table 4: Effect of MENN on Hole cross test in mice

Treatment	Dose(mg/kg)	Number of holes crossed				
		0 min	30 min	60 min	90 min	120min
Vehicle	-	18.40±0.9	11.80±0.66	11.40±0.75	7.80±0.86	2.80±0.86
Diazepam	1mg/kg	21.4±0.91	11±0.94*	3.7±0.79*	2.6±1.04**	4.6±1.04*
MENN	200mg/kg	18.20± 2.54	10.04±0.70	8.9±0.28*	2.2±0.008**	3.2±0.008
MENN	400mg/kg	20.60± 2.62	8.40±0.40	5.8±0.40	1.20±0.37*	1.20±0.37**

Each value is presented as mean ± SEM (n = 5), MENN=Methanol extract *N. nouchali* flower. **p <0.001, *p <0.05 compared with the control group (Dunnett's Test).

Open field test

Open field test of *N. nouchali*200mg/kg and 400mg/kg dose showed shown more significant CNS depressant effect and naturally they fell asleep from its initial value at 30 minute to 120 minutes (Table-5). Initially it was observed that the number of movements of the control group overall increased from 30 minutes to 120 minutes.

Table 5: Effect of *N. nouchali* methanol extracts on Open field test in mice

Treatment	Dose(mg/kg)	Observation				
		0 min	30 min	60 min	90 min	120 min
Vehicle	-	113 ±3.22	106.6±1.69	91.2 ±1.51	87.4 ±1.63	87.4 ±1.63
Diazepam	1mg/kg	102.4±7.04	67.8±5.63*	41.6±3.65	19.6±2.61*	19.6±2.61*
MENN	200mg/kg	81± 5.91	59 ±3.99*	30 ±2.26	15 ±3.50	8 ±0.86**
MENN	400mg/kg	51.4 ± 10.09	47±1.50*	18 ±0.93*	8 ±2.87**	3 ±1.66*

Each value is presented as mean ± SEM (n = 5), MENN=Methanol extract *N. nouchali* flower. **p <0.001, *p <0.05 compared with the control group (Dunnett's Test).

DISCUSSION

Acetic acid-induced writhing method represent pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipid [21]. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics The response is thought to be mediated by peritoneal mast cells [22], acid sensing ion channels [23] and the prostaglandin pathways [24].

The tail immersion test are considered to be selective to examine compounds acting through opoid receptor; the extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain [25, 26]. The extract inhibited both mechanisms of pain, suggesting that the plant extract may act as a narcotic analgesic.

Preliminary phytochemical screening reveals the presence of steroid, alkaloid, tannin, and saponin in the plant extract. So, the observed analgesic activity may be attributed to these compounds. Moreover, recent studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species form phagocytes invading the inflammation sites [27]. There are also reports on the role of tannins in antinociceptive activity [28].

We have detected the presence of carbohydrates, steroids, alkaloids, saponins, and tannins by preliminary phytochemical screening in *N. Nouchali* flower [29]. Scientific investigations reported that plant materials containing flavonoids, phenols and tannins are responsible for analgesic and CNS activity [30-33].

Beside antinociceptive activity, MENN has also been found to have neurobehavioral effects. Both in open field and hole cross test MENN depressed the central nervous system. The locomotor activity of mice gradually decreased during the period of observation (Tables 4, 5) In addition, MENN significantly decreased latency. CNS depressant and sedative drugs potentiate the gamma aminobutyric acid (GABA) mediated post synaptic inhibition via allosteric modification of GABA receptors as well as decrease the sleep latency and increase sleeping time [34-36]. Therefore the significant decrease of locomotion of MENN reveals its potential action on GABA receptors. The $GABA_A$ receptor mediated sedative activity of identified compound phytol [37] provides further evidence of GABAergic mechanism of action of MENN.

CONCLUSION

In light of the findings of the present study, it can be concluded that the plant extract possesses remarkable analgesic and CNS effects. These data lends justifications to the traditional use of the plant in pain and inflammatory disorders. However, further studies are required to understand the underlying mechanism of analgesic and CNS activities and to isolate the active principle(s) responsible for the observed pharmacological effects.

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